

Characteristics and fate of organic nitrogen in municipal biological nutrient removal wastewater treatment plants

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ABSTRACT

The aim of this study was to investigate the occurrence and fate of colloidal and dissolved organic nitrogen (CON and DON) across biological nutrient removal (BNR) activated sludge bioreactors.

Primary and secondary effluent total nitrogen (TN) measurements and component fractionation, CON and DON concentration profiles across BNR bioreactors, and laboratory batch experiments with

the process mixed liquor were carried out at several full-scale BNR plants in northern Poland. The

organic nitrogen (ON) components were divided into high CON, low CON, and DON based on

sequential filtration through 1.2, 0.45 and 0.1 μm pore size filters. The average influent $\text{DON}_{0.1\mu\text{m}}$

(<0.1 μm) concentrations ranged from 1.1 g N/m^3 to 3.9 g N/m^3 and accounted for only 4-13% of

total organic nitrogen. In the effluents, however, this contribution increased to 12-45% (the

$\text{DON}_{0.1\mu\text{m}}$ concentrations varied in a narrow range of 0.5-1.3 g N/m^3). Conversions of ON inside

the bioreactors were investigated in more detail in two largest plants, i.e. Gdansk (565,000 PE)

and Gdynia (516,000 PE). Inside the two studied bioreactors, the largest reductions of the

colloidal fraction were found to occur in the anaerobic and anoxic compartments, whereas an

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26 increase of $\text{DON}_{0.1\mu\text{m}}$ concentrations was observed under aerobic conditions in the last
27 compartment. Batch experiments with the process mixed liquor confirmed that $\text{DON}_{0.1\mu\text{m}}$ was
28 explicitly produced in the aerobic phase and significant amounts of ON were converted in the
29 anoxic phase of the experiments.

30

31 **KEYWORDS**

32 Activated sludge; biological nutrient removal; colloidal organic nitrogen; CON; dissolved organic
33 nitrogen; DON; nitrogen fractionation; nitrogen removal

34

35 INTRODUCTION

36 Biological nitrogen removal (BNR) activated sludge processes are commonly used in municipal
37 wastewater treatment plants (WWTPs) around the world to produce effluents with total nitrogen
38 (TN) concentrations below 10 g N/m^3 . Effluent TN includes total inorganic nitrogen ($\text{NH}_4\text{-N} +$
39 $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$) and total organic nitrogen (TON), which is the sum of dissolved organic
40 nitrogen (DON), particulate organic nitrogen (PON), and colloidal organic nitrogen (CON).
41 Biological nitrogen removal involves transformations and removal of inorganic nitrogen ($\text{NH}_4\text{-N}$
42 and $\text{NO}_x\text{-N}$) by biomass synthesis and sludge wasting, and nitrification ($\text{NH}_4\text{-N}$ oxidation to $\text{NO}_2\text{-}$
43 N and $\text{NO}_3\text{-N}$) and denitrification of $\text{NO}_3\text{-N}$ and/or $\text{NO}_2\text{-N}$ to nitrogen gas. Solid-liquid separation
44 processes, including final clarifiers, sand filters, and membrane filters remove organic nitrogen
45 (ON) contained in suspended solids (as PON) and removable colloidal solids (as CON). Inorganic
46 nitrogen is of primary concern for effluent goals for TN of less than 10 g N/m^3 . With stricter
47 effluent TN permit limits becoming more common in the United States (less than 3.0 g N/m^3),
48 and in some cases in Europe and Japan, the contribution of effluent ON, mainly as DON and
49 CON, has become more important and may account for 30-50% of the effluent TN (WERF,
50 2008). Observed effluent DON contributions vary widely in municipal BNR WWTPs with
51 reported $\text{DON}_{0.45\mu\text{m}}$ concentrations (defined by the fraction passing through $0.45 \mu\text{m}$ pore-size
52 filters) ranging from <2% to as high as 85% of the effluent TN (Pagilla et al., 2006; 2008;
53 Pehlivanoglu and Sedlak, 2004; WERF, 2008).

54
55 Because of the importance of effluent ON in BNR WWTPs addressing low effluent TN
56 concentration goals, understanding the fate of ON in the influent wastewater and across the
57 activated sludge process is of great interest. Influent TN primarily consists of ammonia/ammonium
58 ($\text{NH}_4\text{-N}$) and ON plus none or little in the oxidized inorganic forms. Similar to effluent ON, the
59 influent ON may also be characterized as the sum of PON, DON and CON. Traditionally, a 0.45
60 μm pore-size filter has been used to separate the DON (referred further to as $\text{DON}_{0.45\mu\text{m}}$) from



61 PON in analytical measurements. However, in the work of Makinia et al. (2011), three different
62 pore-size filters were used to separate the ON into PON, CON, and DON fractions. Each physical
63 fraction was further divided into biodegradable and non-biodegradable sub-fractions. This
64 approach resulted in accurate modeling of ON conversions in activated sludge bioreactors
65 (Makinia et al., 2011).

66
67 So far, very little has been done on the fate and characteristics of effluent DON in WWTPs since
68 the early pioneering work of Parkin and McCarty (1981a,b,c), which followed DON in untreated
69 and treated wastewater, and considered sources of DON in the activated sludge effluents,
70 including production during biological treatment. From bench-scale tests they found the lowest
71 effluent DON concentrations at aeration times of 6-9 hours, which corresponded to a 6-10 day
72 aerobic solids retention time (SRT) at the studied WWTP (Palo Alto, California (USA)). Bratby
73 et al. (2008) noted that DON concentration increases through biological treatment, and on the
74 contrary, biological processes in activated sludge systems were identified as a potential method of
75 DON removal (O'Shaughnessy et al., 2006; Pagilla et al., 2006). Factors influencing DON
76 treatment efficiency include SRT, temperature, reactor hydraulics and plant perturbations. Sharp et
77 al. (2009) found that SRT and temperature may impact both PON and DON_{0.45µm} fraction and
78 concentration of effluent DON for a specific plant, but were not the only factors. Studies in
79 several WWTPs in the US and Poland revealed that the effluent concentrations of CON and DON
80 were relatively stable regardless of the influent TN concentrations and process configurations
81 (Pagilla et al., 2008; Sattayatewa et al., 2009b; Sattayatewa et al., 2010). Dignac et al. (2000a)
82 found that the BNR processes can be efficient in removing low molecular weight (LMW) organic
83 matter and DON compounds such as urea, amino acids, and proteins. In contrast, the high
84 molecular weight (HMW) DON is considered to be inert in biological treatment (Gulyas et al.,
85 1995; Dignac et al., 2000a; Pehlivanoglu-Mantas and Sedlak, 2008; WERF, 2008).

86



87 Present knowledge on the characteristics and behavior of CON and DON is still limited and
88 insufficient to estimate BNR process effluent ON concentrations as a function of plant design and
89 influent ON concentration and characteristics. The fate of ON has not been specifically studied in BNR
90 processes and important research questions include (WERF, 2008):

91 - where DON and CON is removed or produced in BNR processes?

92 - what is the effect of BNR process design and configuration (anaerobic and anoxic contact) on
93 effluent DON and CON?

94

95 This paper reports on the results of studies at full-scale BNR WWTPs to address these questions
96 under the Polish conditions which are characterized by very strong municipal wastewater (e.g. TN
97 concentrations are 2-4 times higher compared to the USA). The study evaluated influent
98 wastewater nitrogen characteristics and the fate of nitrogen species across BNR activated sludge
99 bioreactors at eight full-scale WWTPs in northern Poland. Batch experiments were also done at
100 some plants to further investigate nitrogen transformations within different BNR process
101 conditions.

102

103

104 **MATERIALS AND METHODS**

105 **Description of WWTPs**

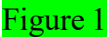

106 All of the WWTPs in the study were designed and operated for both nitrogen and phosphorus
107 removal and varied in size and activated sludge process configurations (Table 1). The effluent TN
108 concentration goal for the four largest facilities, with greater than 100,000 population equivalents
109 (PE), is at the most stringent European Union (EU) standard of 10 g N/m³. The effluent TN
110 concentration goal for the other plants is 15 g N/m³. Design configurations for enhanced
111 biological phosphorus removal (EBPR) are used in all of the facilities except the Elblag WWTP,
112 which has an anoxic-aerobic activated sludge process (MLE) and ferric addition in the primary



113 treatment step for phosphorus removal. The EBPR process configurations are the University of Cape
114 Town (UCT), modified UCT (MUCT), Johannesburg (JHB) and anaerobic-anoxic-aerobic
115 (A_2/O). All the plants were operated over a range of SRTs due to significant seasonal activated
116 sludge temperature fluctuations from 10-22 °C.

117  **Table 1**

118

119 In addition to the primary and secondary effluent ON fractionations, conversions of ON and
120 organic carbon (OC) in BNR activated sludge process steps were investigated in more detail at the
121 two largest plants, Gdansk and Gdynia WWTPs. Process schematics for these plants and the
122 sampling locations are illustrated in  **Figure 1**. More detailed characteristics of those plants can be
123 found elsewhere ( Makinia et al., 2006).

124  **Figure 1**

125

126 **WWTP measurements**

127 ***Influent-effluent analysis.*** Primary and secondary effluent 24-hour, flow-proportional composite
128 samples were collected during ten sampling events between March, 2007 and December, 2008
129 and analyzed for ON and OC fractions. The ON and OC fractions were based on pore-size filter
130 separation and defined as particulate ($>1.2 \mu\text{m}$), "high" colloidal (>0.45 and $<1.2 \mu\text{m}$), "low"
131 colloidal (>0.10 and $<0.45 \mu\text{m}$), and dissolved ($<0.1 \mu\text{m}$). For ON, these are defined as PON,
132 high CON, low CON, and $\text{DON}_{0.1\mu\text{m}}$, respectively. In addition, between January and March, 2009,
133 three additional measurement series were carried out with the Gdansk and Gdynia WWTPs
134 secondary effluent 24-hour composite samples by sequential filtration through 0.1 and 0.015 μm
135 filters to evaluate the effect of ultrafiltration on ON and OC removal.

136

137 ***ON and OC concentration profiles across BNR bioreactors.*** Conversions of non-particulate ON
138 and OC fractions across the Gdansk and Gdynia WWTPs bioreactors were investigated by 5

139 measurement campaigns between November, 2008 and July, 2009. Average colloidal and
140 dissolved ON and OC concentrations in the bioreactors were based on three grab samples (8 AM,
141 11 AM and 2 PM) at the inlet and outlet from the anaerobic, anoxic and aerobic compartments.
142 Sampling point locations at both plants are shown in **Figure 1**.

143

144 **Bench-scale experiments**

145 Bench-scale experiments to evaluate the fate of ON and OC under anaerobic, anoxic, and aerobic
146 conditions were carried out with settled wastewater and return activated sludge (RAS) seed from
147 the Gdansk WWTP (5 tests) and Gdynia WWTP (6 tests) during the same time period as the
148 concentration profile measurements. The experimental apparatus consisted of two parallel 4.0
149 dm³-batch reactors with electrodes for a continuous monitoring of pH, ORP, temperature and
150 dissolved oxygen (DO) and computer control system to maintain DO concentration and
151 temperature around set points. This system also controlled a cyclic measurement of oxygen
152 uptake rate (OUR) in small chambers connected to the main units. During the batch experiments,
153 both reactors were operated in a 3-step sequence of anaerobic (2 h), anoxic (4 h, after addition of
154 KNO₃) and aerobic (6 h) conditions.

155

156 To observe the fate of only the dissolved ON and OC and the effect of PON and CON on effluent
157 DON concentration, reactor 1 (R1) was fed settled wastewater and reactor 2 (R2) was fed pretreated
158 settled wastewater with only DON and dissolved OC constituents (Figure 2). The rapid coagulation-
159 flocculation method by Mamais et al. (1993) based on Zn(OH)₂ precipitation at pH = 10.5 was
160 used to remove particulate and colloidal ON and OC. After removing the colloids and particulates
161 by settling, the pH was adjusted to its original value by adding 6M HCl. The RAS seed was
162 diluted to obtain mixed liquor suspended solids (MLSS) concentration at approx. 2.5-3 kg/m³ in
163 the reactors. The actual MLSS concentrations were measured at the beginning and end of the
164 experiment. A heating/cooling system was set to maintain the batch reactor temperature equal to



165 the actual (current) process temperature in the full-scale bioreactors. After adding the RAS and
166 feed wastewater to the reactors, the mixers were turned on at 180 rpm. Samples of 100-150 cm³
167 were withdrawn at the time intervals shown in **Figure 2**, filtered under vacuum pressure on 1.2
168 µm pore-size filter and then analyzed. The “basic” set of lab analyses comprised NH₄-N, NO₂-N,
169 NO₃-N, COD and PO₄-P measurements, whereas the “full” set of lab analyses included additional
170 TN and total organic carbon (TOC) measurements in 1.2, 0.45 and 0.1 µm pore size filtrates. At
171 the beginning of the anoxic phase (2 hour), potassium nitrate (KNO₃) was added in order to raise
172 the initial concentration of NO₃-N by 20 g N/m³. At the beginning of the aerobic phase (6 hour),
173 the aeration system was turned on and the DO set point was controlled at 6 g O₂/m³.

174  **Figure 2**

175

176 **Analytical methods**

177 The samples were sequentially filtered through membrane filters of different pore sizes including
178 1.2, 0.45 and 0.1 µm pore-size nitrocellulose filters (Billerica MA, USA). The effect of
179 ultrafiltration was investigated with 0.015 µm pore-size polycarbon filters (Whatman, Kent, UK).

180

181 The TOC and TN concentrations were determined using a TOC analyzer (TOC-V_{CSH}) coupled
182 with a TN module (TNM-1) (SHIMADZU Corporation, Kyoto, Japan). Catalytic thermal
183 decomposition/chemiluminescence methods, conformed to the American Society for Test
184 Method's (ASTM) D5176 procedure, are adopted for TN measurement. Samples containing
185 nitrogen are introduced into an oxygen-rich combustion tube with platinum catalyst at a
186 temperature of 720 °C. Bound nitrogen is then converted to nitrogen monoxide (NO), further
187 oxidized to nitrite (NO₂) in the presence of ozone, and is then detected by a chemiluminescence
188 detector. TN concentrations in the range of 0.1 to 4000 g/m³ can be measured.

189

190 The concentrations of inorganic N forms (NH₄-N, NO₃-N and NO₂-N) were determined using



191 Xion 500 spectrophotometer (Dr Lange GmbH, Berlin, Germany). The analytical procedures,
192 which were adopted by Dr Lange, followed the Standard Methods (APHA, 1992). The $\text{DON}_{0.1\mu\text{m}}$
193 and $\text{DON}_{0.45\mu\text{m}}$ concentrations were calculated as a difference between TN after filtration on the
194 appropriate pore size filter (i.e. 0.1 μm and 0.45 μm) and the sum of inorganic N ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$
195 and $\text{NO}_2\text{-N}$) fractions. The CON concentrations were calculated as a difference between TN after
196 filtration on the 1.2 μm pore size filter and the sum of $\text{DON}_{0.1\mu\text{m}}$ and inorganic N fractions.

197

198 RESULTS AND DISCUSSION

199 ON characteristics in primary and secondary effluent

200 The TON accounted for 24-45% of the TN in primary effluent composite samples from 7 of the
201 studied WWTPs (excluding the smallest plant, Koscierzyzna WWTP) and the average primary
202 effluent TON concentrations in those plants ranged from 15.5 g N/m^3 (Elblag WWTP) to 35.0 g
203 N/m^3 (Tczew WWTP) (Table 1). For Koscierzyzna WWTP, the average primary effluent TON
204 concentration and TON/TN ratio were much higher, i.e. 61.0 g N/m^3 and 57%, respectively. The
205 detailed fractionation of primary and secondary effluent ON (including $\text{DON}_{0.1\mu\text{m}}$, low and high
206 CON and PON) is presented in Figure 3. The primary effluent PON fraction (PON/TON) was
207 above 50% for all the WWTPs, with the highest at Koscierzyzna WWTP (78%), at 60-70% for 3
208 WWTPs (Gdansk, Gdynia and Tczew), and near 50% for the remaining four WWTPs (Elblag,
209 Slupsk, Lebork and Kartuzy). For the latter, the colloidal fraction (0.1-1.2 μm) of the TON was
210 very significant (36-44%), whereas it ranged from 17 to 34% for the other WWTPs. The average
211 $\text{DON}_{0.1\mu\text{m}}$ concentrations accounted for only 4-13% of TON and ranged from 1.1 g N/m^3 (Elblag
212 WWTP) to 3.9 g N/m^3 (Lebork WWTP).

213  **Figure 3**

214

215 The average secondary effluent TN concentrations in the WWTPs ranged from 6.5 to 14.1 g N/m^3 . In
216 all the plants, the concentrations of $\text{NH}_4\text{-N}$ were low ($<1.3 \text{ g N/m}^3$) and the dominating form of N



217 was $\text{NO}_x\text{-N}$ (47-73%). The TON portion constituted 23-35% of the effluent TN, and ranged from
218 1.9 g N/m^3 (Tczew WWTP) to 4.3 g N/m^3 (Koscierzyna WWTP). The average contributions of
219 $\text{DON}_{0.1\mu\text{m}}$, CON and PON varied within the ranges of 12-45% ($0.5\text{-}1.3 \text{ g N/m}^3$), 35-44% ($0.7\text{-}1.9$
220 g N/m^3) and 20-43% ($0.4\text{-}1.9 \text{ g N/m}^3$), respectively. The $\text{DON}_{0.1\mu\text{m}}$ and CON concentrations were
221 reduced to a different extent compared to the primary effluent, i.e. on average 56 and 88%,
222 respectively. As a consequence, the average $\text{DON}_{0.1\mu\text{m}}/\text{CON}$ ratio increased from 0.24 in the
223 primary effluent to 0.85 in the secondary effluent. The secondary effluent $\text{DON}_{0.1\mu\text{m}}$
224 concentrations were not (or poorly) correlated to any ON form in the primary effluent (the highest
225 correlation, $R^2 = 0.29$, was found with respect to the primary effluent CON concentration). In
226 contrast, a good correlation ($R^2 = 0.55$) was found between the secondary effluent $\text{DON}_{0.45\mu\text{m}}$
227 concentrations and primary effluent CON.

228
229 Ultrafiltration ($0.015 \mu\text{m}$) after $0.10 \mu\text{m}$ filtration of the Gdansk and Gdynia WWTP effluents
230 resulted in similar and relatively small amounts of $\text{DON}_{0.10\mu\text{m}}$ and dissolved organic carbon
231 ($\text{DOC}_{0.10\mu\text{m}}$) removal. The average reductions in $\text{DON}_{0.10\mu\text{m}}$ and $\text{DOC}_{0.10\mu\text{m}}$ at both plants were 10-
232 13% and 2-3%, respectively (Table 2). Thus, it can be assumed that the $0.10 \mu\text{m}$ filtration
233 provides a reasonable approximation of the DON. The DON consists of both low and high
234 molecular weight (LMW and HMW) compounds as shown by Pehlivanoglu-Mantas and Sedlak
235 (2008) for which almost all of a secondary effluent DON passed through a 10 kDa filter (0.005
236 μm). About half of the DON passed through a 1 kDa filter and represents LMW substances,
237 which may include free and combined amino acids and synthetic organics. Nitrogen may also be
238 present in humic acids at the 3-10 kDa size and higher molecular weight range. In the earliest studies
239 on the characteristics of DON in secondary effluents, Keller et al. (1978) and Parkin and McCarty
240 (1981c) reported the percentage of LMW nitrogen compounds ($<1.8 \text{ kDa}$) in the range of 50-66%.
241 Bratby et al. (2008) also noted a dominance of LMW DON from a previous study, in which 78% of
242 a secondary effluent DON was at a molecular size of 1.0 kDa or less.



243  **Table 2**

244

245 The average primary and secondary effluent TOC concentrations varied from 125-320 g C/m³
246 and 9.4-17.8 g C/m³, respectively (detailed data not shown). Biological treatment consistently
247 increased the DON/DOC ratio of the wastewater for both conventional (0.45 µm pore size) solids
248 separation filtration and the 0.1 µm pore size approaching the true dissolved filtrate (Figure 4). In
249 general, the smaller pore size filtrate had a higher ON/OC ratio suggesting that the colloids in the
250 pore size range of 0.45-0.1 µm contain proportionally higher amounts of ON than OC. A similar
251 switch of the DON/DOC ratio was also found by Dignac et al. (2000b) in a conventional activated
252 sludge pilot plant treating municipal wastewater (SRT = 10 d). The elemental analysis after
253 electro dialysis (ED) revealed that the ON/OC atomic ratio increased from 0.07 (influent
254 wastewater) to 0.12 (ozonated sludge) and 0.14 (conventional sludge). Lower degradation rates
255 for ON would increase the secondary effluent DON/DOC ratio, as observed the full scale
256 facilities evaluated here, which agrees with Parkin and McCarty (1981a) reporting that the
257 observed degradation rate of DON_{0.45µm} in raw wastewater was less than 50% of the degradation
258 rate of dissolved COD. The authors attributed this change to the presence of heterocyclic nitrogen
259 compounds, such as nucleic acid bases, which can yield high N/COD ratios. Westerhoff and Mash
260 (2002) noted that the DON/DOC ratios are >0.1 in receiving waters affected by wastewater
261 discharges, agricultural activity or high algae productivity, whereas the DON/DOC ratios are
262 <0.025 in receiving waters not affected by WWTP effluents or other sources of N pollution.

263  **Figure 4**

264

265 The effluent ON concentrations observed in the northern Poland WWTPs are within the range
266 reported for municipal WWTPs in the USA Chesapeake Bay region, where very stringent effluent
267 TN limits of 3 g N/m³ have been set to help control eutrophication. This study's WWTP data and

268 USA effluent DON data (WERF, 2008; Sattatayewa et al., 2009b) were plotted in Figure 5 to
269 show the probability distributions of the DON concentrations. WERF (2008) summarized the
270 reported effluent DON data from 32 BNR facilities across the USA and found that effluent DON
271 concentrations (without defining the filter pore-sizes used at each plant) varied in the range of 0.1-
272 2.8 g N/m³ with the 50 and 90 percentile values of 1.2 and 2.1 g N/m³, respectively. The same
273 figure also presents the distribution of effluent DON data from a recent study (Sattatayewa et al.,
274 2009b) for 7 US BNR plants. The average DON_{0.45µm} concentrations in three samples collected at
275 each plant ranged from 0.6 to 1.4 g N/m³ and the 50 and 90 percentile values were 0.9 and 1.7 g
276 N/m³, which is significantly lower in comparison with the results of the 32 plants presented by
277 WERF (2008). It should be noted that at two WWTPs (Blue Plains, DC and Stamford, CT), also
278 included in the review of WERF (2008), the DON_{0.45µm} concentrations found by Sattatayewa et al.
279 (2009b) were lower by 0.6 and 0.9 g N/m³, respectively. For comparison, very similar values of the
280 50 and 90 percentiles, i.e. 0.9 and 1.3 g N/m³, were obtained for the Polish plants but with respect
281 to the DON_{0.1µm} concentrations.

282  **Figure 5**

283
284 Similar ranges of ON concentration were also reported for other US WWTPs. In the study of
285 Pehlivanoglu-Mantas and Sedlak (2008), the DON_{0.2µm} concentration (in the samples passed
286 through 0.2 µm pore-size filters) ranged from 0.7 to 2.1 g N/m³ in the effluents from three WWTPs
287 (conventional and N removal activated sludge processes, and a biofilm system). A very similar
288 range (0.5-2.0 g N/m³) was reported by Sattatayewa et al. (2010) in four BNR plants regardless of
289 the effluent TN concentrations (<5 to 14 g N/m³). At another three WWTPs (conventional and N
290 removal activate sludge processes), Westgate and Park (2010) found the effluent TON
291 concentrations in the range of 0.9-1.7 g N/m³ (7-29% of TN) at very low effluent VSS
292 concentrations (1.3-6.1 g/m³).

293



294 The effluent DON consists of compounds which are difficult to remove or produced during
295 biological treatment (Pehlivanoglu-Mantas and Sedlak, 2008). Despite the variety of ON-
296 containing compounds detected in municipal WWTP effluents, most of the compounds could not
297 be identified with available methods. The authors estimated the contributions of some groups of
298 the compounds as: 1) 10-20% - dissolved free and combined amino acids (these compounds are
299 most likely produced during biological treatment since amino acids and proteins are readily
300 biodegradable), 2) <5% ethylenediaminetetraacetic acid (EDTA), and 3) 10% - humic substances
301 originating from the drinking water sources.

302

303 A relatively low amino acid fraction in municipal WWTP effluent DON of only about 10% was
304 also noted by Parkin and McCarty (1981c) and Dignac et al. (2000a). Furthermore, Dignac et al.
305 (2000a) concluded that some of the difficulties in identifying DON with common analytical
306 methods may be due to the presence of complex structures, which are concentrated during the
307 biological treatment as a result of the resistance to microbial degradation. Parkin and McCarty
308 (1981c) suggested that a combination of nucleic acid degradation products, nucleic acid bases,
309 and heterocyclic nitrogen compounds could account for up to 25% of the effluent DON_{0.45µm}.
310 Westgate and Park (2010) found a strong correlation between protein-N and ON concentrations in
311 the effluent wastewater. The protein-N constituted a substantial fraction (approximately 60%) of
312 effluent ON. However, the authors admitted that this value may be overestimated due to two
313 potential sources of the errors: (1) humic substances that are present in effluents can interfere with
314 the Lowry protein measurement, (2) inaccuracy of the ON determination by subtracting inorganic
315 forms from the TN. It should also be noted that the analysis was performed on non-filtered
316 samples (see above)).

317

318 **Fate of DON/CON in activated sludge bioreactors**

319 *Measurement of DON/CON concentration profiles inside bioreactors.* Examples (fall and spring



320 measurement series) of $\text{DON}_{0.1\mu\text{m}}$ and CON profiles in the full-scale bioreactors at the Gdansk
321 and Gdynia WWTPs are presented in **Figure 6**. During the measurements, the sum of CON and
322 $\text{DON}_{0.1\mu\text{m}}$ concentrations in the bioreactor effluent remained in a narrow range, i.e. 1.9-2.3 g N/m³
323 (Gdansk WWTP) and 2.1-2.4 g N/m³ (Gdynia WWTP). For comparison, the corresponding
324 average concentrations during the comprehensive survey were 1.7 g N/m³ (standard deviation
325 0.32 g N/m³) and 2.2 g N/m³ (standard deviation 0.86 g N/m³) at the Gdansk and Gdynia
326 WWTPs, respectively.

327

328 At the Gdansk plant, the primary effluent $\text{DON}_{0.1\mu\text{m}}$ concentrations ranged from 1.1 to 2.2 g N/m³ and
329 these values are comparable to the results of the comprehensive survey (average concentration 1.8
330 g N/m³, standard deviation 2.42 g N/m³). For modeling N conversions in that plant, **Makinia et al.**
331 **(2011)** assumed that only 10% of the primary effluent DON (<0.2 g N/m³) and CON (>1.0 g
332 N/m³) was non-biodegradable, which ultimately accounted for approximately 50% of the sum of
333 effluent DON and CON concentrations. For comparison, **Parkin and McCarty (1981a)** estimated
334 that under optimal conditions (SRT = 6-10 d), inert substances from the influent wastewater
335 represented 60-80% of the effluent $\text{DON}_{0.45\mu\text{m}}$, whereas the remaining portion was produced during
336 activated sludge treatment by the biomass.

337

338 Low concentrations of $\text{DON}_{0.1\mu\text{m}}$ were observed in all the sampling points along the MUCT
339 bioreactor. A minor increase in the last (aerobic) compartment was compensated by a reduction of
340 the “low” (0.1-0.45 μm) colloidal subfraction. The effluent $\text{DON}_{0.1\mu\text{m}}$ concentrations ranged from
341 0.3-1.1 g N/m³, which is relatively low when compared to the results of the comprehensive survey
342 (average concentration 1.2 g N/m³, standard deviation 0.71 g N/m³). The largest reductions of
343 both colloidal subfractions were found to occur in the anaerobic and anoxic compartments, which
344 is substantially affected by influent dilution and by the recirculated mixed liquor. In the anaerobic
345 compartment, the concentrations of “low” (0.1-0.45 μm) and “high” (0.45-1.2 μm) colloidal



346 subfractions decreased by 2.7-3.0 g N/m³ and 2.1-2.5 g N/m³, respectively. In the anoxic
347 compartment, the corresponding maximum reductions were 0.8-1.0 g N/m³ and 1.9-2.0 g N/m³.

348

349 At the Gdynia plant, relatively high primary effluent DON_{0.1µm} concentrations (2.4-3.5 g N/m³) were
350 observed in comparison with the results of the comprehensive survey (average concentration 2.2 g
351 N/m³, standard deviation 1.00 g N/m³). The DON_{0.1µm} concentrations decreased to 0.6-0.9 and 0.3-
352 0.7 g N/m³, respectively, in the anaerobic and anoxic compartment. However, similar to the Gdansk
353 WWTP, an increase in DON_{0.1µm} concentration and decrease in the “low” CON subfraction were
354 observed under aerobic conditions in the last compartment. The highest reductions of the “low” and
355 “high” CON subfractions were observed in the anaerobic compartment (by 3.2-4.3 g N/m³) and
356 anoxic compartment (by 1.7-2.4 g N/m³), respectively.

357

358 With regard to colloidal and dissolved OC, the dominant primary effluent fraction (75-80%) at
359 both plants was DOC, which is very similar to the results of the comprehensive survey (see above).
360 The DOC concentrations primarily decreased in the anaerobic compartment (by 50-70%), whereas
361 the DOC reduction in the entire bioreactors reached 75-85%. The colloidal fraction was reduced
362 in the anaerobic compartment and then stabilized. After that, no significant transformations
363 between the two colloidal subfractions (“low” and “high”) were observed.

364  **Figure 6**

365

366 **Sattayatewa et al. (2009a)** carried out similar measurements of ON concentration profiles in a
367 full-scale 4-stage Bardenpho bioreactor. The primary effluent DON_{0.45µm} concentrations were low,
368 ranging from 0.84 to 1.37 g N/m³ with the average value 1.11 g N/m³. The results showed ON
369 release in the primary anoxic compartment and no ON release in the first aerobic compartment of
370 the activated sludge process. With regard to the DON concentrations determined in the samples
371 after pretreatment using the coagulation-flocculation method of **Mamais et al. (1993)** (DON_{ff}), a

372 decreasing trend was observed along the treatment train until the first anoxic compartment and
373 then remained stable in the last three compartments (first aerobic/second anoxic/second aerobic).
374 In the bioreactor effluent, $\text{DON}_{0.45\mu\text{m}}$ and DON_{ff} were essentially identical. In the follow-up study
375 (Sattayatewa et al., 2010), however, the effluent $\text{DON}_{\text{ff}}/\text{DON}_{0.45\mu\text{m}}$ in four BNR bioreactors
376 ranged from 0.76 to 0.95 suggesting a significant contribution of the “low” CON subfraction to
377 the effluent ON.

378

379 ***Determination of DON/CON behavior in bench-scale experiments.*** The average CON and
380 $\text{DON}_{0.1\mu\text{m}}$ concentrations (\pm standard deviations) for all the experiments are summarized in Table
381 3, and Figure 7 illustrates an example of the behavior of CON and $\text{DON}_{0.1\mu\text{m}}$ profiles measured at
382 the Gdansk and Gdynia WWTPs plants during the summer testing. In all the cases, $\text{DON}_{0.1\mu\text{m}}$ was
383 explicitly produced in the aerobic compartment of the anaerobic/anoxic/aerobic process. For the
384 Gdansk batch tests, the $\text{DON}_{0.1\mu\text{m}}$ concentration increases ranged from 0.6-1.9 (average 0.9) g
385 N/m^3 when fed pre-settled wastewater influent (reactor 1) and ranged from 0.2-1.3 (average 0.7) g
386 N/m^3 when fed coagulated-flocculated wastewater influent (reactor 2). Corresponding results for
387 the Gdynia WWTP were concentration increases of 0.3-1.1 (average 0.5) g N/m^3 (reactor 1) and
388 0.4-1.6 (average 0.8) g N/m^3 (reactor 2), respectively. These findings are in accordance with the
389 observations in the full-scale activated sludge process in which $\text{DON}_{0.1\mu\text{m}}$ concentrations also
390 increased in the aerobic compartment. Furthermore, in order to evaluate if the increase under
391 aerobic conditions was statistically significant, unpaired t-tests with unequal variances were
392 performed on two data sets ($\text{DON}_{0.1\mu\text{m}}$ concentrations at the end of anoxic and aerobic
393 period/zone). The calculated p-value, which is the probability of observing equal concentrations
394 for these sampling points, varied at both studied plants in the range of 0.008-0.009 and 0.05-0.09,
395 respectively, for the batch tests and full-scale measurements. Such low p-values, especially for the
396 batch tests, suggest that the examined increase was statistically significant.


397



398 It should also be noted that the behavior of inorganic N forms ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$) was
399 similar in the batch tests with the process mixed liquor from both WWTPs (Makinia et al., 2009).
400 However, complete nitrification could not be achieved due to high initial $\text{NH}_4\text{-N}$ concentrations
401 ranging from 35 to 40 g N/m^3 , and $\text{NO}_2\text{-N}$ accumulated to a peak concentration of approximately 9
402 g N/m^3 in one test at the Gdansk WWTP. During the other tests, the peak $\text{NO}_2\text{-N}$ concentrations
403 ranged from 2 to 4 g N/m^3 . In contrast, no $\text{NO}_2\text{-N}$ accumulation was observed during the
404 measurements either in the aerobic compartments of the full-scale bioreactors or in the secondary
405 effluents during routine samplings by the plant operators.

406

407 In contrast to the full-scale measurements, the CON profile behaviors in the anaerobic and anoxic
408 phases were different for the two WWTPs. During the Gdansk WWTP batch experiment, an
409 apparent production of DON from CON hydrolysis occurred in the anoxic phase. This finding
410 was derived based on the observation that lower amounts of CON were utilized and higher
411 amounts of DON were produced in reactor 2 (containing exclusively the soluble fraction)
412 compared to reactor 1 (containing all the fractions). These results are consistent with the full-scale
413 measurements at that plant. On the other hand, the batch test CON behavior was the opposite in
414 the Gdynia batch tests, with only a slight decrease in CON (reactor 1) or increase (reactor 2)
415 (Table 3).

416  **Table 3**

417  **Figure 7**

418

419 Until now, the only laboratory experiments evaluating the behavior of ON in activated sludge
420 were reported by Parkin and McCarty (1981b,c). Parkin and McCarty (1981c) carried out a series
421 of eight aeration batch tests (up to 72 h) and the average ultimate $\text{DON}_{0.45\mu\text{m}}$ concentration was
422 1.4 (± 0.46) g N/m^3 . Based on the N mass balance, the authors estimated that 52% of it was

423 recalcitrant (resistant to biological transformations) from the influent wastewater sources, 19%
424 was produced from biomass endogenous decay in the activated sludge process (this portion is a
425 function of the biomass concentration and SRT, 16% was in equilibrium between that sorbed to
426 biomass and that in the liquid (this portion is independent of the biomass concentration and
427 temperature, but dependent on cultural characteristics) and about 13% could be further degraded.
428 Furthermore, the authors noted that increasing the activated sludge SRT could either degrade
429 further influent $\text{DON}_{0.45\mu\text{m}}$ or it could also raise $\text{DON}_{0.45\mu\text{m}}$ via biomass endogenous respiration.
430 The compounds released during biomass decay were defined as “poorly biodegradable”.
431 Furthermore, **Parkin and McCarty (1981b)** observed peak $\text{DON}_{0.45\mu\text{m}}$ concentrations (0.1 to 0.6 g
432 N/m^3) during the initial 6 hours of aeration batch tests regardless of initial substrate, MLSS and
433 $\text{NH}_4\text{-N}$ concentrations, and a substrate type. The authors attributed that excretion of DON to
434 concentration gradients, starvation conditions, addition of exogenous substrate, and changes in
435 phase and rate of growth. Excreted $\text{DON}_{0.45\mu\text{m}}$ was subsequently utilized by the microorganisms,
436 leading to a minimum $\text{DON}_{0.45\mu\text{m}}$ concentration after 4 to 8 hours. Starvation conditions then
437 prevailed, and a gradual increase in $\text{DON}_{0.45\mu\text{m}}$ concentration occurred due to microorganism
438 decay. This finding is consistent with the observations of the bench-scale experiments at the
439 Gdansk and Gdynia WWTPs where minimum $\text{DON}_{0.1\mu\text{m}}$ concentrations were observed at the end
440 of the anoxic phase (after 6 hours from the beginning of the experiment).

441

442

443 CONCLUSIONS

444 From this study in the 8 BNR WWTPs, the following conclusions can be derived:

- 445 ■ The average primary effluent $\text{DON}_{0.1\mu\text{m}}$ concentrations ranged from 1.1 $\text{g N}/\text{m}^3$ to 3.9 $\text{g N}/\text{m}^3$
446 and $\text{DON}_{0.1\mu\text{m}}$ accounted for only 4-13% of TON concentrations which varied in a wide range
447 from 15.5 to 61 $\text{g N}/\text{m}^3$. The fraction of $\text{DON}_{0.1\mu\text{m}}$ increased to 12-45% of the effluent TON

448 following BNR activated sludge treatment, but the average secondary effluent $\text{DON}_{0.1\mu\text{m}}$
449 concentrations ranged from only 0.5 to 1.3 g N/m³ which implies the biodegradability of a
450 major portion of the primary effluent $\text{DON}_{0.1\mu\text{m}}$.

451 ■ The relatively narrow range of secondary effluent $\text{DON}_{0.1\mu\text{m}}$ concentrations was observed
452 despite high variations in the size of the studied plants (37,000-565,000 PE), biological
453 process configuration employed (UCT, MUCT, JHB, A₂/O, MLE) and operating parameters
454 (SRT = 9-34 d). Furthermore, the secondary effluent $\text{DON}_{0.1\mu\text{m}}$ concentrations were not (or
455 poorly) correlated to any ON form in the primary effluent (the highest correlation, $R^2 = 0.29$,
456 was found with respect to the primary effluent CON concentration). In contrast, a good
457 correlation ($R^2 = 0.55$) was found between the secondary effluent $\text{DON}_{0.45\mu\text{m}}$ concentrations
458 and primary effluent CON.

459 ■ The secondary effluent CON fraction accounted for 43 to 78% of the non-particulate ON (<1.2
460 μm) with the remainder as $\text{DON}_{0.1\mu\text{m}}$. Ultrafiltration with 0.015 μm pore size filters had only a
461 minor effect on further reductions of the effluent $\text{DON}_{0.1\mu\text{m}}$.

462 ■ The largest reductions of the CON fractions were found to occur in the anaerobic and anoxic
463 compartments of the studied bioreactors, whereas an increase of $\text{DON}_{0.1\mu\text{m}}$ concentrations was
464 observed in the aerobic compartment. During batch experiments with process mixed liquor and
465 primary effluent wastewater, $\text{DON}_{0.1\mu\text{m}}$ was consistently produced in the aerobic phase.

466

467

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474 Seattle.

475

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558



559 **Table 1. BNR WWTPs configuration, average flowrate, SRTs, and plant influent and**
 560 **effluent TN concentrations for 2007-2008 (based on the routine operating data)**
 561
 562

Facility	Size	Average flowrate	SRT	Bioreactor configuration	Average TN (2007-08)	
					Influent	Effluent
	PE	m ³ /d	d		gN/m ³	gN/m ³
Gdansk	565,000	81,000	21-31	MUCT	81.2	11.6
Gdynia	516,000	56,000	14-27	JHB	82.5	12.9
Elblag	181,000	36,000	15-22	MLE	66.9	5.6
Slupsk	180,000	19,000	18-29	UCT	73.9	8.4
Tczew	70,000	8,900	19-34	A ₂ /O	84.5	6.8
Lebork	56,000	7,200	18-25	JHB	80.7	10.9
Kartuzy	47,000	3,200	9-24	UCT	97.9	14.0
Koscierzyna	37,000	3,200	12-29	JHB	113.0	10.3

563

564

565 **Table 2. DON and DOC before and after 0.10 μm filtration and ultrafiltration of the**
 566 **secondary effluents at the Gdansk and Gdynia WWTPs**
 567
 568

Gdansk WWTP					
Sample	Unit	Test 1	Test 2	Test 3	Average
DON (<0.1 μm)	g N/m ³	1.88	2.10	1.99	1.99
DON _{UF} (<0.015 μm)	g N/m ³	1.77	2.04	1.59	1.80
DOC (<0.1 μm)	g C/m ³	17.0	17.5	10.2	14.9
DOC _{UF} (<0.015 μm)	g C/m ³	16.6	17.2	9.4	14.4
Gdynia WWTP					
Sample	Unit	Test 1	Test 2	Test 3	Average
DON (<0.1 μm)	g N/m ³	1.52	1.63	1.05	1.40
DON _{UF} (<0.015 μm)	g N/m ³	1.26	1.39	1.00	1.22
DOC (<0.1 μm)	g C/m ³	16.0	10.7	9.9	12.2
DOC _{UF} (<0.015 μm)	g C/m ³	15.8	10.4	9.6	11.9

569

570

571 **Table 3. Average CON and DON concentrations during the 3-phase**
 572 **anaerobic/anoxic/aerobic batch experiments at the Gdansk and Gdynia WWTPs**
 573
 574

Gdansk (average ± standard deviations from 5 experiments) (T = 12.8-16.8 °C, MLVSS = 1.17-3.04 kg/m ³)						
	Reactor 1 (without pretreatment)			Reactor 2 (with pretreatment)		
Sample	DON	CON	Total	DON	CON	Total
Start	0.6 ± 0.21	3.3 ± 1.34	3.9 ± 1.53	0.6 ± 0.56	2.1 ± 0.84	2.7 ± 1.20
Anaerobic	0.8 ± 0.52	3.3 ± 1.04	4.1 ± 1.43	0.8 ± 0.42	2.1 ± 0.84	2.9 ± 1.15
Anoxic	0.7 ± 0.33	2 ± 0.33	2.7 ± 0.50	0.2 ± 0.08	2.1 ± 0.88	2.3 ± 0.91
Aerobic	1.6 ± 0.55	2.1 ± 0.76	3.7 ± 0.38	0.9 ± 0.42	1.7 ± 1.00	2.6 ± 0.82

Gdynia – (average ± standard deviations from 6 experiments) (T = 13.1-17.9 °C, MLVSS = 1.21-2.57 kg/m ³)						
	Reactor 1 (without pretreatment)			Reactor 2 (with pretreatment)		
Sample	DON	CON	Total	DON	CON	Total
Start	0.8 ± 0.38	2.4 ± 0.84	3.2 ± 1.00	0.7 ± 0.32	1.8 ± 0.93	2.5 ± 0.78
Anaerobic	0.8 ± 0.45	2.1 ± 0.77	2.9 ± 1.03	0.9 ± 0.51	1.8 ± 0.56	2.7 ± 0.90
Anoxic	0.6 ± 0.28	1.9 ± 0.8	2.5 ± 1.03	0.6 ± 0.17	2.3 ± 1.17	2.9 ± 1.12
Aerobic	1.1 ± 0.58	2.2 ± 0.62	3.3 ± 1.12	1.4 ± 0.70	2.4 ± 1.18	3.8 ± 1.38

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579 **Figure 1. Schematic layouts of bioreactor compartments: (a) Gdansk MUCT process, (b)**
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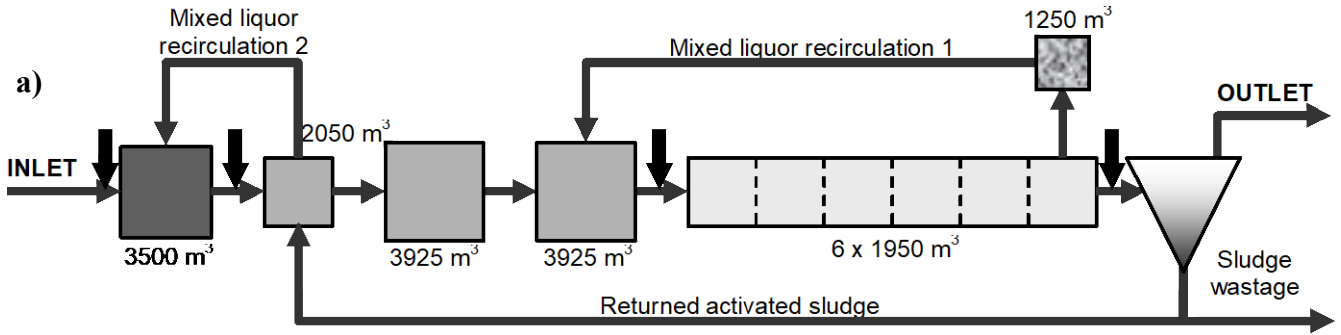
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593 **wastewater, (d) settled wastewater after coagulation-flocculation**

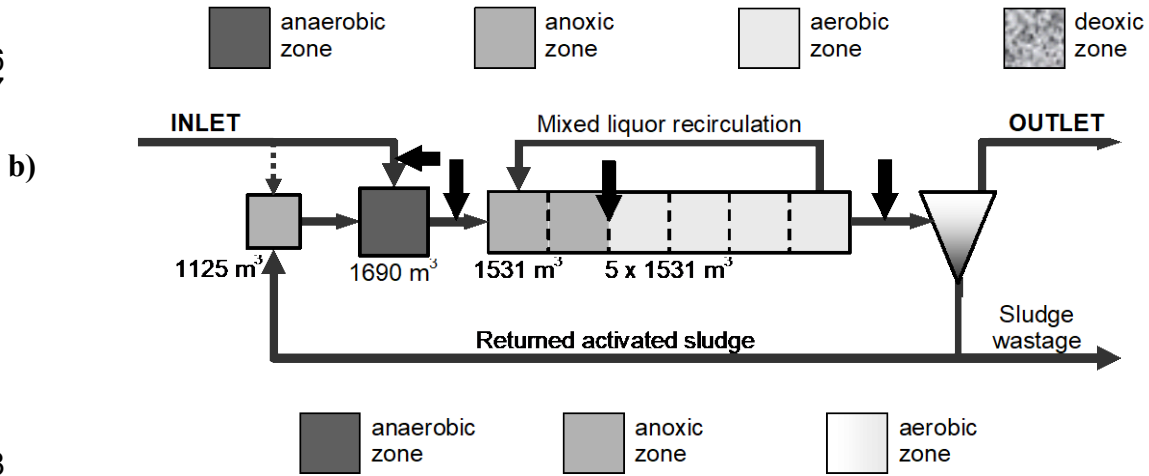
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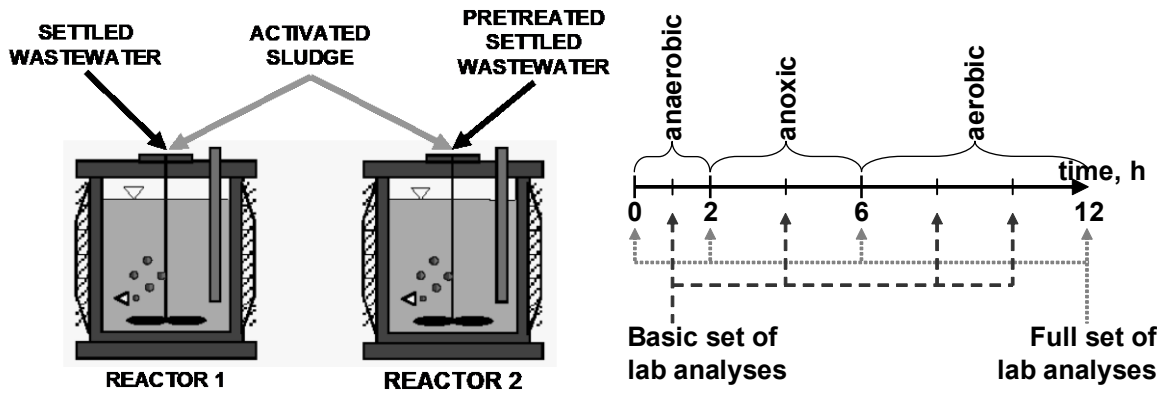
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Figure 1. Schematic of BNR system bioreactor compartments: (a) Gdansk MUCT process, (b) Gdynia JHB process (black arrows show sampling locations)

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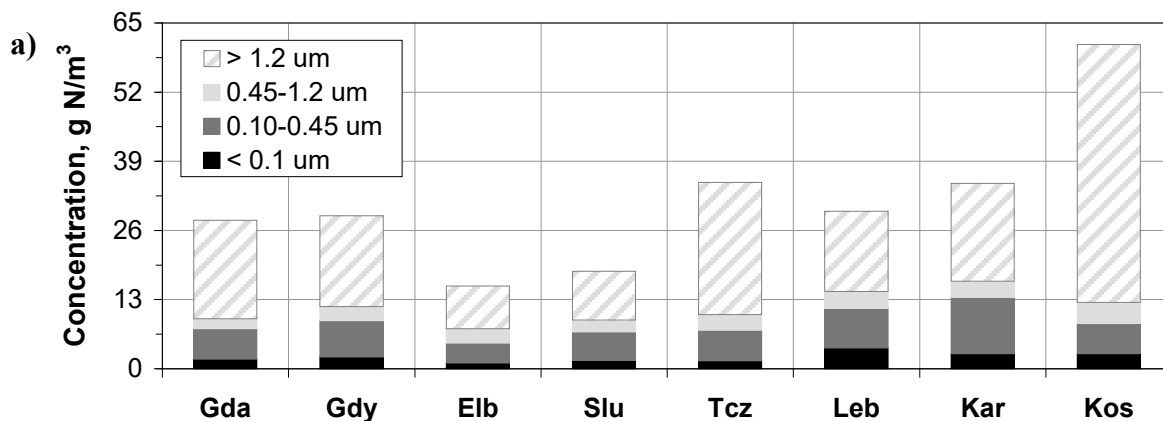
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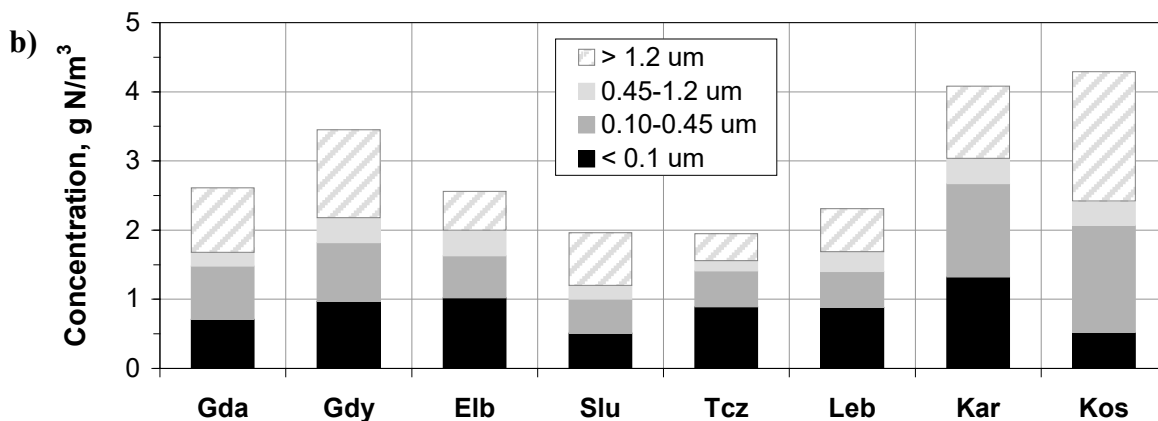
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614 **parallel batch reactors (parameters measured in the basic and full sets are described in**
615 **the text)**

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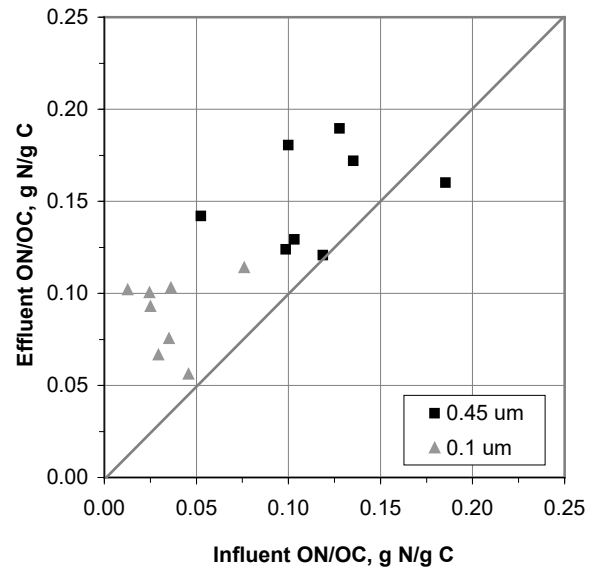
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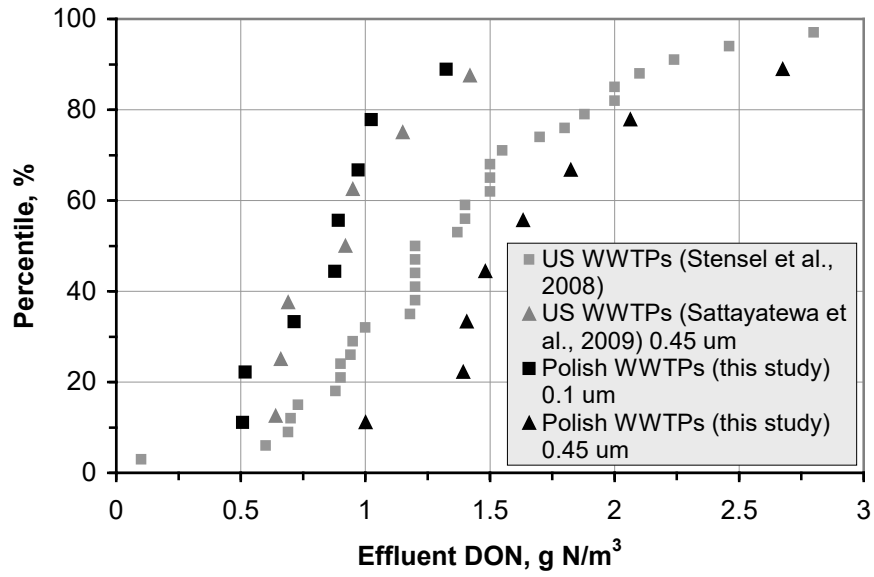
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Figure 3. Primary and secondary effluent ON fractions in the 8 studied BNR WWTPs



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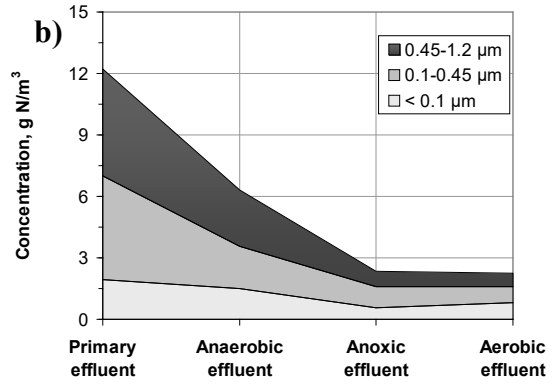
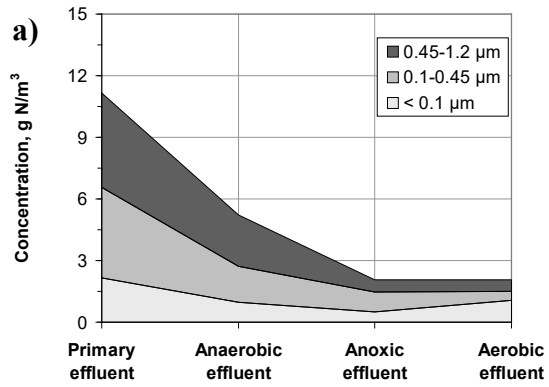
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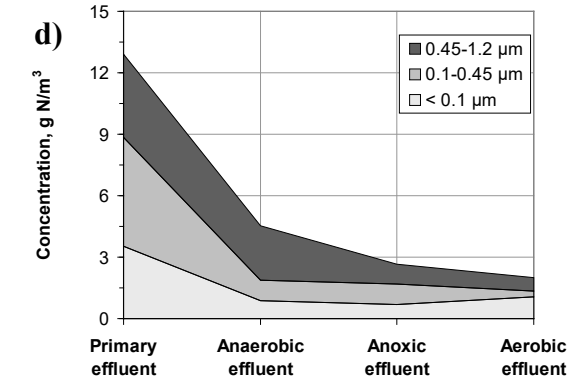
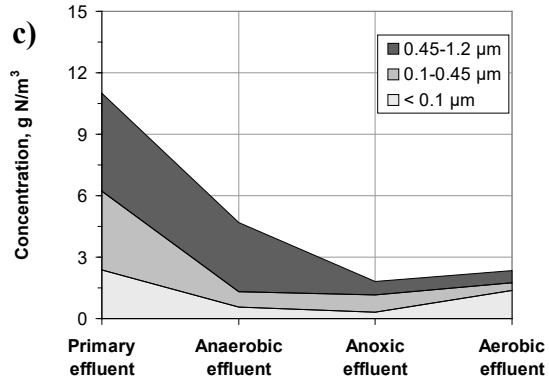
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Figure 5. Probability distributions of secondary effluent DON concentrations

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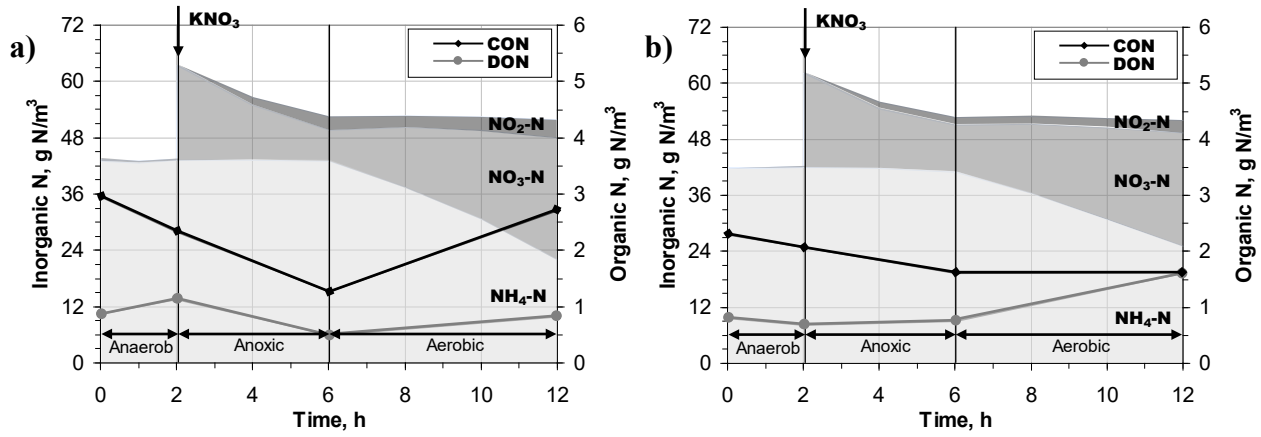
651 **Figure 6. Average concentration profiles of DON and CON in the full-scale bioreactors**

652 **(fall and spring study sessions) at the Gdansk WWTP (a-b) and the Gdynia WWTP (c-d)**

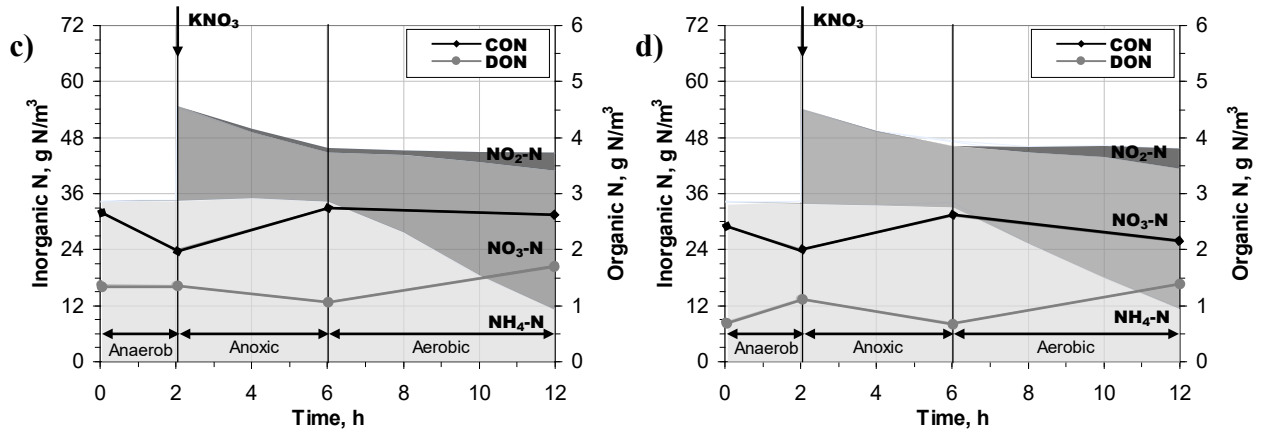
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Figure 7. Behavior of nitrogen compounds during 3-phase batch tests with the full-scale process biomass from the Gdansk WWTP: (a) settled wastewater, (b) settled wastewater after coagulation-flocculation, and the Gdynia WWTP: (c) settled wastewater, (d) settled wastewater after coagulation-flocculation